Survey of Banana endophytic fungi isolated in artificial culture media from an applied viewpoint


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Interactions among populations of beneficial organisms and pathogens do not occur in isolation. Instead, interactions within the entire community will determine the success or failure of a biological control strategy. Therefore, the complexity of the entire pathosystem must be considered [14]. One must also consider the area in which the interaction between the pathogen and control agent is going to take place. If the interaction takes place at the infection site, antibiosis and competition for nutrients or space may be important mechanisms in biocontrol.

Searching non-chemical strategies for the control of crop diseases is of considerable interest because of environmental and health concerns about the widespread use of synthetic pesticides [18]. The use of microorganisms as antagonists of plant pathogens could be a solution. However there is still an important lack of knowledge of the diversity, distribution or influence of these fungi in the development and/or prevention of plant diseases [7]. Our aim in this study is to survey the fungal endophytic community of banana plants in order to generate the knowledge required to further generate efficient biocontrol tools.

Material and Methods

Sampling were carried out in Dwarf naine banana (Musa acuminate, AAA group, Cavendish subgroup) fields of the Canary Islands (table 1): La Palma (site 1: Tazacorte, site 2: Punta Llana) and Tenerife (site 3: Buenavista, site 4: Los Gigantes). Plants with no external symptoms of disease were selected for sampling fragments of the corm. At the moment of extracting the corm fragment an internal inspection was performed in order to prove that the plant did not presents symptoms of banana weevil.

A surface sterilization method [20] was used in order to suppress epiphytic microorganisms from the plant samples. Thus, branch fragments were first washed with sterile water, afterwards immersed in 75% ethanol for 1 min, followed by an immersion in 5% sodium hypochlorite for 5 min, again in 75% ethanol for 30

Many microfungi are able to live in living plant tissues. In contrast to plant pathogens and parasites the so called endophytic fungi do not cause obvious disease symptoms in their hosts [25].

Endophytes show a remarkable chemical diversity of secondary metabolites and therefore possess an important biotechnological potential in medicine, agriculture and industry [24, 12, 13, 23]. Current research on endophytes is aiming at a better understanding of the ecology and evolution of these organisms, their impact on ecosystem composition and plant communities as well as their unique natural products [1].

One of the main threats of bananas throughout the world is Fusarium wilt [26]. The disease is caused by Fusarium oxysporum f.sp. cubense (Foc) through a systemic vascular infection [22]. Foc, like most pathogenic isolates of Fusarium oxysporum, colonizes vascular tissue, leading to the disruption of water translocation to the shoot [3].

Options to control Fusarium wilt are limited as chemical control is inefficient and on the market suitable resistant cultivars are lacking [21].

Biological control of a plant disease can be accomplished by direct or indirect interactions of the pathogen populations and the control agent [14]. It has been suggested that endophytic microorganisms play a key role in the host-pathogen interactions prior to the triggering of the disease [8, 16]. The mechanisms capable to prevent and/or restrict the development of plant pathogens have been studied in some endophytes and these are: induction of systemic resistance and expression of defence genes against the attack of certain pathogens in their hosts [2, 11]; production of secondary metabolites that inhibit the growth of other fungi and competition with plant pathogenic fungi for space and nutrients within the host [9, 17].

Isolation of fungal endophytes of banana corm resulted in 15 colonies from 4 different genus taxa (Aspergillus, Penicillium, Fusarium and Chaetonium). Endophytic fungi isolated were evaluated in vitro to observe antagonistic effects against the Fusarium wilt. Three endophytic fungi (2 Aspergillus spp. and 1 Penicillium spp.) inhibited mycelia growth of the pathogen.

Endophytic fungi, banana, biocontrol, antagonism

Key words

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seconds and lastly were washed again two times with sterile distilled water. After this process, plant material was dried on sterile blotting sheet under sterile conditions, excised in pieces of 2 cm and cut in half with a sterile scalpel, segments were placed in potato dextrose agar (PDA) Petri plates. Plates with the plant segments were incubated at 22°C in the dark for 3–20 days and observed daily. When fungal outgrowth from the plant tissues occurred, mycelia fragments were subculture on fresh PDA medium. Eventually, when an endophyte was acquired in pure culture it was preserved and identified.

**Identification of fungal isolates**

Prior to taxonomic identification, a preliminary visual inspection was made in order to avoid the selection of identical strains arising from the same plant stand. Among these, morphological identification was carried out based on macroscopic and microscopic appearances. Macroscopic descriptions were made from 5 days-old colonies grown in PDA and incubated at 22°C in darkness. Microscopic slide preparations of fungal mycelium were stained with cotton blue and mounting media consisted in a mixture of resin and xylene (1:1).

Mycelia is scraped using a sterile scalpel from 1-or 2-week-old PDA fungal cultures and sequenced by the Genomics Service of the University of La Laguna (Tenerife, Spain). Amplification of the internal transcribed spacer (ITS) region was carried out using the universal eukaryotic primers of ITS1 (5′-TCCGTAAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′).

Sequence-based identifications were realized by searching with FASTA algorithms the EMBL/Genbank database of fungal nucleotide sequences. When the homology between a strain sequence and a sequence in GenBank was greater than 99%, the match was accepted at the species level. The genus alone was accepted when similarity fell between 95 and 99%.

**Fungal strains preservation**

Long-term conservation methods were used in order to preserve all the fungal isolates, these consisted in keeping, at -30°C and -70oC, fungal fragments grown on PDA in vials with one of these cryoprotectants: DMSO (10%) or glycerol (18%).

**Antagonism assay of endophytic fungi against Fusarium oxysporum f.sp. cubense**

Dual culture technique was used as screening method to find endophytic fungi that produce metabolites which inhibit *Fusarium oxysporum f.sp cubense* growth in vitro. Potato dextrose agar medium was selected for dual culture as it favors growth of plant pathogenic fungi. The plates were incubated at 22°C in darkness during 3–7 days and observed daily. Antagonism was expressed following a modified scale proposed by Neville & Webster (1995) [15].

- A1. Pathogenic fungi grows over the endophyte
- A2. Endophyte grows over the pathogen
- B. Deadlock (when both fungi contact each other and stop growing)
- C. Deadlock at a distance or with a change of mycelia color or aspect at the margin of contact.
- D. Presence of inhibition zone at the point of interaction.

Our interest is on those interactions of type D because the inhibition zone may indicate than the endophyte might be producing some substance which inhibits growth of pathogen mycelia.

### Results and Discussions

**Isolation and fungal identification**

Endophytic fungi were isolated from healthy banana tissues collected from two locations in two of the Canary Islands (table 1): La Palma (site 1: Tazacorte, site 2: Punta Llana) and Tenerife (site 3: Buenavista, site 4: Los Gigantes). The incubation of banana fragments, resulted in the isolation of 15 endophytes which belong to 4 different genus (*Aspergillus, Penicillium, Chaetonium and Fusarium*).

![Table 1](image-url)

Table 1: Sampling sites and taxa isolated banana endophytic fungi

- **Sampling sites**
  - Tazacorte
  - Punta Llana
  - Buenavista
  - Los Gigantes

- **Abreviated code**
  - P1
  - P2
  - P3
  - P4
  - P5
  - P6
  - P7
  - P8
  - P9
  - P10
  - P12
  - P15
  - T1
  - T2
  - T3

- **Isolated taxa**
  - Aspergillus sp.
  - Penicillium sp.
  - Aspergillus sp.
  - Chaetonium sp.
  - Fusarium sp.
  - Fusarium oxysporum
  - Aspergillus sp.
  - Penicillium sp.
  - Aspergillus flavus
  - Aspergillus oryzae
  - Fusiarm sp.
  - Penicillium radicum
  - Aspergillus oryzae

Screening for an antagonistic effect of endophytic fungi on the growth of Foc was performed by dual culture of both fungi in Petri dish. Isolates P3 (*Aspergillus sp.*), T2 (*Penicillium radicum*) and T5 (*Aspergillus sp.*) showed type D interaction with an inhibition zone of a width > 5mm. These results are interesting as a first approach yet it is not possible to affirm that interactions observed in these bioassays take place inside the banana plant.
Conclusions

The present study describes the composition of the endophytic fungal communities within the plant tissues of banana from Canary Islands. All the associated mycota belongs to ascomycetes fungi which is in agreement with previous reports [4, 5, 9]. It has been pointed out that the isolation of ascomycetes is favored by the sampling methods used, since the sporulating and fast-growing species generally belong to asexual ascomycetes [19, 6]. Antibiosis, wherein a microorganism produces a metabolite which inhibits the growth of the pathogen, may be most important when the interaction is restricted to infection sites. For the antibiotic to play an important role, however, it would have to be produced at the proper time and at high enough levels to protect the infection site from the pathogen. The present survey is a first approach to the endophytic community of banana plants, this knowledge is fundamental when trying to reveal the interactions that take place inside the plant between fungal pathogen, the host and other microorganisms. Also the comprehension of the pathosystem is essential in order to develop effective biocontrol schemes.

Natural product biopesticides and biocontrol agents are important tools for achieving a productive and sustainable agriculture [10]. Further research must be done to understand the interactions occurring between fungal community inhabiting plant tissues. These studies can help the search of new biopesticides sources and biocontrol agents.

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References


